An overview of the hepatitis viruses

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Readers are invited to use this article as a self-assessment exercise and to update their knowledge.

HISTORICAL PERSPECTIVE

Liver disease and jaundice have been known and described since antiquity. The first written evidence that jaundice was recognized as infectious was in the eighth century in a letter from Pope Zecharias to St Boniface, and epidemics of jaundice have long been associated with war, famine and other catastrophic events. The first unmistakable outbreak of blood-borne viral hepatitis was recorded in 1885 among personnel of a Bremen shipyard who had been vaccinated against smallpox using human lymph; the vaccine was implicated as the cause of the epidemic. Despite this elegant piece of work and the evidence of other epidemiologic studies during the latter part of the nineteenth century and the first decades of this, it was generally believed that jaundice, commonly called catarrhal jaundice, was caused by an obstruction of the common bile duct with a mucus plug. A few lone voices, such as MacDonald in 1908, postulated a viral etiology for the disease, but the conventional view of acute hepatitis as an obstructive disease was not seriously challenged until World War II, when a number of classical studies in the UK and the USA using human volunteers established beyond doubt the viral and infectious nature of the disease and distinguished the two main routes of transmission, feco-oral and parenteral.

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In the decades immediately following the war, considerable money and effort were spent in investigating the viral etiology of hepatitis, but none were successful in identifying a candidate agent in cell culture. Major advances in understanding the modes of transmission, incubation times, and duration of viremia and of viral shedding of the two types of viral hepatitis were made through studies in experimentally infected primates. Further human experiments in and observations on mentally handicapped children, and residents in institutions where viral hepatitis was endemic, proved that what became known as hepatitis A and B viruses (HAV, HBV) were immunologically distinct and that a long-term carrier state existed for HBV.

The breakthrough came in the early 1960s by pure chance; Blumberg and his coworkers discovered an antigen in the serum of an Australian aborigine that was later found to be the coat protein of HBV. Blumberg was interested in the genetic polymorphism of serum proteins and was studying the precipitin reactions between antibodies in the serum of multiply transfused patients and antigens in a panel of sera from different geographical regions by simple two-dimensional immunodiffusion. This 'Australia antigen' was not at first associated with hepatitis, but because of the frequency with which it was found in the serum of patients with acute leukemia, it was suggested that it might be of value in the early diagnosis of this disease. Sera from patients with Down's syndrome were examined because of the high risk of leukemia in this group, and one such patient seroconverted to Australia antigen positive at the same time as developing acute hepatitis. Thus was the link made between the presence of the antigen in serum and HBV. Subsequently, in 1974, the virus of hepatitis A was discovered by Feinstone and coworkers and then identified as a picornavirus. Since then (see below) a series of other hepatitis viruses have been discovered.

THE HEPATITIS ALPHABET

At the time of the discovery of HBV surface antigen (HBsAg), post-transfusion jaundice was the most serious complication of the use of blood and blood products. Viral hepatitis was also a growing problem in hemodialysis, with a case mortality between 6% and 28%, and in the early 1970s HBsAg screening of blood donors and staff and patients in hemodialysis units was introduced. These early screening tests for HBsAg were insensitive but the technological development of new assays was rapid and by the end of the decade highly sensitive commercial immunoassays were available at relatively low cost. In-house and commercial assays for the diagnosis of hepatitis A by detection of IgM antibody were developed at this time, initially using fecal antigen from human cases or experimentally infected marmosets, but later using cell-culture antigen. Following the introduction of routine HBsAg screening of blood donors it rapidly became obvious that there was another type of parenteral hepatitis. The consensus of various studies was that 90% of all cases of post-transfusion jaundice and 'transaminitis' were caused by this agent or agents [1], and in the USA the risk of 'non-A, non-B hepatitis' (NANBH) following a transfusion was estimated to be between 7% and 12% [2].

Hepatitis delta virus (HDV) was discovered in 1977 by immunofluorescence studies on liver biopsies [3]. HDV is a defective virus which cannot replicate without the help of HBV. Infection occurs either as a simultaneous co-infection with HBV or by super-infection of an HBV carrier. However, intensive work over more than a decade using conventional immunologic or virus isolation methods failed to identify any other agent of parenteral NANBH until eventually hepatitis C virus (HCV) was identified by cloning viral nucleic acid fragments from serum and the development of immunoassays for antibodies to recombinant proteins derived from these fragments [4]. Serologic surveys have shown that HCV is responsible for the majority of cases of post-transfusion NANBH and is implicated in some cases of community-acquired sporadic hepatitis [5,6]. Acute epidemic hepatitis in adults associated with the consumption of sewage-contaminated food or water is common in India and Africa, and it had been assumed that this was hepatitis A; but re-examination of stored sera in the 1980s showed that this was not usually the case. This enteric form of NANBH, now termed hepatitis E, was instead recognized as the commonest cause of acute hepatitis in adults in India, Asia and Africa [7]. Hepatitis E virus (HEV) is a calicivirus-like agent.

The classification of hepatitis viruses after HEV is very confused, and new candidate hepatotrophic viruses are still being discovered as a result of the application of novel molecular biological techniques. It is possible that not all of these agents will definitely be accepted as true hepatitis viruses. A form of sporadic hepatitis was described in 1991 based on examination of thin sections of liver biopsies [8]. This was tentatively called hepatitis G, but this has since been dismissed. The infection was characterized by the presence of giant multinucleated syncytial hepatocytes containing intracytoplasmic structures with the appearance of paramyxovirus nucleocapsids. A 60-nm particle seen in the livers of patients with fulminant hepatitis, and in subsequent graft necrosis following liver transplantation, was originally proposed as hepatitis F virus. This may be the same as two other viruses, GBV-C and HGV, the RNA of which has been found in the serum of multi-transfused patients by amplification of cDNA fragments [9–13]. These two agents are currently grouped together as hepatitis G virus (HGV). Meanwhile the best current candidate for hepatitis F virus is a 27–37-nm particle found in stool extracts by electron microscopy from cases of sporadic non-parentally acquired NANBH which are infectious for rhesus monkeys by intravenous inoculation [14].

The confusion that now exists in the field of hepatitis viruses will eventually be sorted out by the application of molecular biology through techniques such as nucleic acid sequencing and by standard serologic methods using recombinant proteins or synthetic peptide antigens in surveys of the prevalence of antibodies to the various agents in different human populations. A provisional listing is given in Table 1.

Table 1 A provisional listing of hepatitis viruses

	Mode of spread	Size of particle (nm)	Nucleic acid	Family
Hepatitis A	Feco-oral	25–30	ss-RNA	Picornaviridae
Hepatitis B	Parenteral	42	ds-DNA	Hepadnaviridae
Hepatitis C	Parenteral	?	ss-RNA	Flavivirus-like
Hepatitis D	Parenteral	35	ss-RNA	?
Hepatitis E	Feco-oral	27–34	ss-RNA	Calicivirus-like
Hepatitis F	Feco-oral	27–37	ds-DNA	?
Hepatitis G	Parenteral	60	ss-RNA	Flavivirus-like

MULTIPLE CHOICE QUESTIONS

In each of the numbered questions, at least one, and up to five, of the individual entries are correct. (The answers are at the end of this article.)

1. The liver is the primary target organ for the following viruses

(a) Hepatitis A virus True/False
(b) Cytomegalovirus True/False
(c) Hepatitis B virus True/False
(d) Hepatitis C virus True/False
(e) Yellow fever virus True/False

2. Hepatitis A and B

(a) Both have an incubation period of about 2 weeks True/False
(b) Can both be transmitted by blood or blood products True/False
(c) Cause an illness with similar clinical symptoms True/False
(d) Always cause jaundice True/False
(e) Both cause a chronic infection True/False

3. Acute hepatitis A

(a) Is predominantly a disease of young children True/False
(b) Is associated with poor socio-economic conditions True/False
(c) In developed countries is usually travel associated in adults True/False
(d) Is a significant hazard for healthcare workers True/False
(e) May be prevented by vaccination True/False

4. Hepatitis B

(a) Has a uniform worldwide carriage rate of about 10% True/False
(b) May be transmitted across the placenta in utero True/False
(c) Is a sexually transmitted disease True/False
(d) Destroys infected hepatocytes by lysis True/False
(e) Is the major cause of primary hepatocellular carcinoma True/False

5. Hepatitis B

(a) Is an important cause of nosocomial infection True/False
(b) Is a preventable disease True/False
(c) Vaccination confers lifelong immunity in 99% of subjects True/False
(d) Immune response to vaccine directed against coat of virus True/False
(e) May be prevented by use of normal human immune globulin True/False

6. Hepatitis C

(a) Viral RNA is detectable in serum much sooner than antibody True/False
(b) Is transmitted sexually at same frequency as hepatitis B True/False
(c) Initiates a chronic infection in a high proportion of cases True/False
(d) May be transmitted from mother to infant True/False
(e) Can be successfully treated with interferon-α True/False

7. Delta hepatitis

(a) Virion RNA encodes for a single protein True/False
(b) Virion coat is hepatitis B surface antigen True/False
(c) Viral genome resembles that of certain plant viruses True/False
(d) Superinfection of a hepatitis B carrier leads to delta carriage True/False
(e) Co-infection with HBV increases the risk of fulminant disease True/False

8. Hepatitis E

(a) Causes large epidemics with thousands of cases True/False
(b) Fecal contamination of drinking water is major source of infection True/False
(c) Highest attack rate is in children True/False
(d) Is endemic in all countries of the world True/False
(e) Acute infection has a high mortality rate True/False

COMMENTS

Question 1

Many viruses are capable of replication in hepatocytes, resulting in characteristic symptoms of acute infection of the liver, and in particular jaundice. Most viruses capable of systemic infection only invade the body after replication in epithelial cells, and few human virus species are primarily hepatotrophic. HAV is an enterovirus in the family Picornaviridae and is distantly related to poliovirus. The primary site of replication is in epithelial cells lining the gastrointestinal tract; the liver is infected via the bloodstream. Cytomegalovirus may be isolated from a variety of body fluids, including saliva, urine, breast milk, genital secretions and blood. The virus, though cytopathic, is of low pathogenicity,

and following acute infection the virus becomes latent in polymorphonuclear leukocytes. The most common route of infection is by oral contact, primary virus replication taking place in the epithelial cells lining the salivary ducts. From the bloodstream the virus commonly infects kidneys and liver but specific symptoms are usually absent. During severe disseminated disease, evidence of infection may be seen in virtually all organs, but this is unusual. Hepatitis B and C viruses both replicate primarily in hepatocytes. There is evidence that they also replicate in peripheral blood lymphocytes, but is not clear how important this is in pathogenesis of disease [15,16]. Yellow fever virus is a member of a family of viruses causing hemorrhagic fevers and diseases of the central nervous system; the majority are transmitted via biting insects. In yellow fever the target organ is the liver, virus replication causing hepatocellular damage. Renal involvement is common, with acute tubular necrosis, and there is considerable evidence that mononuclear leukocytes play an important role in the disease.

Question 2

Studies in primates and humans in the 1940s and early 1950s established the length of incubation for hepatitis A and B. Hepatitis A has an average incubation of 30 days, with a range of 14 to 45 days. For hepatitis B the incubation period is much longer, with a mean of 70 days and a range of 30 to 180 days. In both diseases the length of incubation is proportional to the dose of infectious virus; in particular, the extremely long incubation that is sometimes seen with hepatitis B may reflect a small infecting dose. Hepatitis A has a viremic phase which precedes the appearance of jaundice. Although virus is present in the bloodstream for only a few days during this time, blood and blood products are capable of transmitting the infection.

Both hepatitis A and B start with non-specific prodromal symptoms including headache, malaise, lethargy, anorexia, nausea, vomiting and diarrhea. The icteric phase follows after a few days and normally lasts for a week or two. Patients often start to feel better before jaundice has resolved. Some patients experience a serum sickness-like syndrome at the onset of the illness, with fever, rash and arthralgia. This is most often seen with hepatitis B but also occurs with hepatitis A. Jaundice is not universal. About 50% of HBV infections are anicteric, and hepatitis A is usually asymptomatic in young children. Hepatitis A is generally an acute, self-limiting infection which is normally less severe and of shorter duration than hepatitis B. The mortality rate in otherwise fit and healthy patients is very low indeed. Hepatitis B usually has a more insidious onset and there are often relapses during convalescence; the mortality rate is about 1%. Fulminant hepatitis, which is characterized by encephalopathy and is usually fatal, is a rare complication of hepatitis in developed countries; it is more often seen with hepatitis B, though it can occur with hepatitis A. A characteristic of HBV is its ability to initiate a long-term carrier state in a significant proportion of infected patients. Chronic infection is unknown with HAV, but rarely, in susceptible individuals, it can act as a trigger for autoimmune chronic hepatitis [17].

Question 3

Hepatitis A occurs worldwide as an endemic disease. In developing countries there is a high incidence of infection. The virus is transmitted among small children, and most people are immune by their teens. In the developed countries the incidence of infection is falling due to improvements in public health and sanitation and with increasing wealth [18–20]. Many adults in the industrialized countries have no natural immunity and are susceptible if they come into contact with a case of hepatitis A or consume contaminated food or water. Consequently, acute hepatitis A in adults from developed countries is associated with travel to areas of high endemicity, although infection could be avoided by fairly simple precautions [21]. However, even in a developed country such as the UK, community outbreaks of hepatitis A are common, with transmission by person-to-person contact [22]. Typically, cases of acute hepatitis are seen in older children and adults who are in contact with children, such as teachers and relatives. Food- and water-associated outbreaks have also been reported, and shellfish are a particularly common source of infection [23,24]. Certain occupations may carry an increased risk of hepatitis A, e.g. sewage workers [25,26]. Hepatitis A is not a major hazard for healthcare workers; few cases of acute hepatitis A require hospitalization and, since spread is feco-oral, normal hygienic precautions are sufficient to prevent transmission.

A formalin-inactivated HAV vaccine prepared from marmoset liver was produced in 1978 and shown to produce protective antibody in human volunteers. Large-scale vaccine production became possible once the virus had been grown in vitro in cell culture in 1979, although priority at that time was being given to HBV vaccine development. In 1986 a formalin-inactivated cell-culture-grown HAV vaccine was shown to be safe and effective in adult human volunteers. Manufacturers in many countries joined in the development of HAV vaccines, and commercial vaccine became available in the UK in 1992 [27]. In efficacy trials, 100% seroconversion was seen in susceptible volunteers after two doses of vaccine given

2 to 4 weeks apart [28]. A third, booster dose was recommended after 6 months. An improved version of the vaccine was introduced in 1994. The primary course consists of a single dose with a booster dose after 6 to 12 months for extended immunity. Most human isolates of HAV belong to a single serotype with less than 10% genotype sequence divergence, and vaccine should therefore give almost universal protection. Strains of the virus with up to 20% sequence variability have been isolated from different parts of the world but these represent only limited amino acid changes. One of these divergent genotypes appears to be circulating in a part of the world where HAV is hyperendemic, but the epidemiologic significance of this phenomenon is unknown [29].

Question 4

Worldwide there may be as many as 300 million carriers of HBV. The carriage rate varies widely. In the UK, northern Europe, the USA, Canada, Australia and New Zealand it is 0.1% or lower, in the countries bordering the Mediterranean and the Middle East it is between 2% and 5%, whilst in sub-Saharan Africa, the Indian sub-continent and Central and South America it is between 5% and 15%. The highest rates are seen in the countries of South East Asia, the Far East and the Pacific islands, where the rate may be as high as 20% of the adult population.

In countries with a low carriage rate, higher rates are seen in people in aboriginal populations in ethnic groups originating in areas where there is a high carriage rate and in patients with risk factors such as a history of multiple transfusion, dependence upon blood products, immune impairment, injecting drug use and sexual activity with frequent partner change, particularly sex between men. Vertical and sexual transmission are the two major routes of infection of this virus. Horizontal spread by exchange of body fluids between people who live in close physical proximity is also important and may explain some cases of sporadic hepatitis B where specific risk factors are absent. Intrauterine infection only occurs where there is a very high level of infectious virus in the bloodstream of the mother, as in acute infection during pregnancy and in a small proportion of carriers [30]. Transmission from mother to infant is mostly perinatal or occurs during the first months of life, possibly via breast milk.

Hepatitis B viral replication does not result in lysis of the infected hepatocyte. Replication is intense during the prodromal phase of the acute illness, and this is accompanied by production of 'e' antigen and excess surface antigen, which are both actively secreted by the cell into the extracellular spaces and thence into the blood. The 'e' antigen is coded for by part of the gene that codes for the core protein of the virus, which has two promoter sites. Core antigen is not secreted by the cell and is not found in the blood other than as a component of mature virus particles; antibody to the core, initially IgM and later IgG, is consistently detected in acute cases, carriers and those who have recovered from the infection. During the normal course of the disease, viral replication falls off and 'e' antigen is replaced in the blood by its corresponding antibody. Ultimately all replication may cease, surface antigen disappear and, after a very variable time interval, antibody to surface antigen become detectable. Most carriers have only low levels of viral replication, characterized by the presence of 'e' antibody in blood, but not antibody to surface antigen (anti-HBs). It has been suggested that clearance of infected hepatocytes from the liver is a function of a cell-mediated immune response to viral nucleocapsid and surface proteins. High serum levels of the cytokines tumor necrosis factor and interleukin-2 and -6 have been observed in the prodromal phase of acute infection and they may have a role in hepatocyte injury [31]. Chronic carriage develops in approximately 70% of neonates and very young children, in whom the immune response is immature, and in adults who are immunosuppressed. Five per cent or fewer of acutely infected healthy adults become carriers and symptomatic infection rarely results in carriage. It has been concluded that establishment of the carrier state is the result of an impaired cell-mediated response. Possible mechanisms include failure of the major histocompatibility complex to invoke a response and an absence of endogenous interferon production [32]. The protein product of the viral X gene, which is now thought to function as a transcriptional transactivation protein, may play a determining part in the range of pathogenesis of HBV infection, from fulminant hepatitis to asymptomatic carriage [33].

The consequence of chronic infection is often chronic liver disease [34], and the viral genome may become integrated into the host-cell genome, ultimately leading to primary hepatocellular carcinoma (HCC). The probability that a carrier of HBV will develop hepatocellular cancer is 100-fold greater than for non-infected individuals, and regions with high rates of carriage have the highest incidence of HCC.

Question 5

Before the advent of screening for HBsAg, hepatitis B was a frequent and sometimes serious complication of blood transfusion, treatment with blood products, hemodialysis and organ transplantation. There are still a few cases of post-transfusion hepatitis B each year because a screening assay has failed to detect surface

antigen in a patient who has infectious virus in their blood.

In some cases of hospital-acquired infection the patient is infected during surgery or some other invasive procedure from a healthcare worker, usually a surgeon, who is a carrier of HBV [35–38]. The UK guidelines stipulate that all healthcare workers involved in exposure-prone procedures where there is a possibility of transmission of HBV should be vaccinated. Those who fail to respond after three doses and a booster should be tested for other HBV markers. Anyone who is HBV surface antigen positive should be tested for 'e' antigen and antibody, and if they are 'e' antigen positive should not be permitted to perform exposure-prone procedures [39,40].

A small percentage of carriers have a precore stop mutation in the gene coding for the core protein of the virus. The effect of this mutation is to prevent translation of the protein, and 'e' antigen is not secreted into the blood even where there is a high level of virus replication [41,42]. These rare carriers may also transmit HBV. Another nosocomial source of HBV is cross-infection between patients.

Perinatal infection can be prevented by immune prophylaxis with hyperimmune immunoglobulin and vaccine at birth. This will significantly reduce the number of carriers in the community in the future and is the most effective way to reduce the morbidity and mortality that result from HBV infection [43–46]. In countries where there is a very low prevalence of HBV, e.g. the UK, screening of pregnant women for HBsAg and prophylaxis for their infants is the single most effective intervention to prevent hepatitis B.

All HBV vaccines consist of surface antigen proteins. The first vaccines were prepared from human plasma, treated to inactivate infectious virus. Second generation vaccines were recombinant proteins made by cloning and expressing the surface antigen gene, in yeast in the case of Engerix B. These vaccines contained only the S epitope of the surface antigen protein, whereas the plasma-derived vaccines had also contained the pre-S1 and pre-S2 epitopes. New recombinant vaccines are in development with all epitopes represented.

Clinical trials of vaccine were carried out in healthy young adult volunteers and levels of greater than 10 IU/L were seen in almost 100% following three doses of vaccine. Very little difference was seen between the plasma-derived and recombinant vaccines. Because of the diversity of individual responses to vaccine, the time-dependent decrease in antibody level seen in all studies and the possibility of errors in quantitative antibody determination, it was recommended that patients with a level less than 100 IU/L should be boosted. The UK policy is to give further booster doses after 5 years or earlier if the level of antibody is found to have fallen below 100 IU/L. In the USA it is assumed that the immune memory is adequate for long-term protection, even where the level of circulating antibody has fallen below 10 IU/L [47]. There are few studies on the persistence of long-term immunologic memory with recombinant vaccines; in one in vitro study, immunologic memory for HBsAg was seen in B-lymphocytes derived from vaccinees who had lost their antibody but not in B-cells from non-responders [48]. Vaccine response declines with age and is better in women than in men [49].

Our experience in nearly 6000 healthcare workers, tested within 2 months of completion of a course of three doses, was that 8% had less than 10 IU/L and a further 13% between 10 and 100 IU/L anti-HBs. In 420 subjects given a booster because they had less than 100 IU/L after three doses and where all antibody tests were performed within 2 months after vaccination, we found that 25% had less than 10 IU/L and 42% more than 100 IU/L after the fourth dose; 56% of those with less than 10 IU/L after three doses still had less than 10 IU/L after four doses. A proportion of vaccine non-responders are chronic carriers or have serologic evidence of past infection; it has also been suggested that non-responsiveness might be due to latent infection [50].

Normal human immunoglobulin made from donated blood in industrialized countries contains only low levels of antibody to HBV. In the UK, specific hyperimmune immunoglobulin is prepared from the plasma of donors with high levels of anti-HBs, and preparations typically contain 100,000 IU/L. Human anti-HB monoclonal antibodies manufactured in transformed human mononuclear cells might be used in the future for passive prophylaxis [51]. Before the advent of HBV vaccine, anti-HB immunoglobulin was used for prophylaxis following exposure to HBV and for treatment of the infants of HBsAg-positive mothers; two doses were necessary at an interval of 1 month. In babies a third dose was often given at 6 months. It is now used in combination with vaccine for prophylaxis of non-vaccinated personnel and vaccine non-responders who have been exposed to a known HBV-positive person, and for infants of 'e' antigen-positive mothers; only a single dose is necessary. Long-term immune prophylaxis of HBsAg-positive patients with cirrhosis or fulminant hepatitis who undergo liver transplantation has been found to reduce the incidence of reinfection of the graft [52].

Question 6

Acute hepatitis C is often asymptomatic and sporadic cases are rarely diagnosed at the time of infection. Much that is known about the disease has been learned from

studies of post-transfusion jaundice, although there is no significant difference between this and the sporadic disease. Specific antibody response is delayed for several weeks following exposure to the virus, but viremia is detectable much sooner, as shown by a positive polymerase chain reaction (PCR) following extraction and reverse transcription of viral RNA to cDNA.

Studies of both post-transfusion and sporadic hepatitis C have shown that the patient becomes chronically infected in more than 50% of cases [53]. There is some evidence that antibody levels remain high in patients with chronic infection but fall with time in those who clear the virus. Chronic infection is associated with raised though often fluctuating serum transaminase levels. Examination of liver biopsies reveals histopathologic evidence of chronic liver disease, and cirrhosis is a long-term consequence, though the rate of progression is usually very slow [54–56]. There is evidence that HCV has an etiologic role in mixed cryoglobulinemia, HCV RNA being detected in peripheral blood mononuclear cells and bone marrow cells [57].

Transfusion with blood or treatment with blood products are major risk factors for hepatitis C, and seroprevalence of HCV antibodies is high in multiply-transfused patients and hemophiliacs [58,59]. Studies of sporadic hepatitis C and seroprevalence studies in risk groups point to injecting drug use as a major risk factor. Our own studies in patients attending genitourinary medicine clinics in the UK show an HCV antibody seroprevalence of 58% in injecting drug users compared to 2.8% in homosexual men and 0.7% in subjects of both sexes with multiple sexual partners. Serologic studies of sexually promiscuous groups show that the rate of seropositivity is significantly higher than in controls, indicating some sexual transmission, but at much lower frequency than with hepatitis B [60–65]. One study in hemophiliacs failed to show any sexual transmission but noted that this cohort might be more likely than others to practice protected intercourse to avoid HIV transmission [66]. In another study the chance of being HCV positive was three times higher in the sexual partners of patients with chronic hepatitis than in those whose HCV-infected partners had no biochemical evidence of liver disease and five times higher than in controls [67].

The evidence for vertical transmission of HCV from mother to infant is far from conclusive. Some recent studies of anti-HCV in mother-and-infant pairs have failed to demonstrate persistence of passively acquired antibodies even in the infants of HIV-positive mothers [68]. In contrast, in a study using nested PCR, viremia was found in five out of eight HIV-positive women and in four of their offspring [69]. One child had persistent viremia, chronic elevation of transaminases and remained anti-HCV positive; the other three children had intermittent viremia but lost their HCV antibodies. Lack of persistence of anti-HCV in the child is possibly insufficient evidence from which to conclude that vertical transmission has not occurred.

Interferon-α is increasingly being used to treat patients with chronic HCV infection, inducing remission in up to 50% of patients [70,71]. The response is inversely correlated with pretreatment serum levels of HCV RNA and severity of liver disease, and is influenced by HCV genotype [72]. It has been suggested that interferon selects resistant subtypes of the virus which replicate once treatment is withdrawn [73]. Measurable persistence of HCV RNA in serum is a better indicator of response to interferon than the serum transaminase levels.

Question 7

HDV is a unique animal virus and bears a striking resemblance to a number of plant pathogens known as viroids [74]. The HDV RNA is a single-stranded circular molecule, approximately 1700 nucleotides long, with an unbranched rod-shaped secondary configuration caused by pairing of up to 70% of the bases. Complementary antigenomic RNA is detected in infected hepatocytes, serving as a template for synthesis of progeny RNA genomes. The largest open reading frame of the antigenomic RNA codes for the single viral-encoded protein, delta antigen. In the maturing virion, the RNA genome is encapsulated in the delta antigen, which is then enclosed in a coat of surface antigen provided by the helper HBV.

Fulminant hepatitis is seen significantly more often in co-infected patients than in those infected with HBV alone, especially when infection with HBV and HDV is simultaneous. Serum HBV DNA and HBsAg levels are lower, suggesting that HDV suppresses HBV replication. Chronic carriage is rare following simultaneous infection, but when it occurs progression to chronic liver disease is much more rapid than with HBV carriage alone. Acute hepatitis is seen in 50% to 70% of HBV carriers who are superinfected with HDV. Persistent levels of IgM antibody to delta antigen following superinfection, which are not seen with co-infection, are evidence of ongoing replication of HDV, with the possibility of more rapid progression to chronic active hepatitis and cirrhosis.

Question 8

In developing countries the majority of the population have been infected with HAV in early childhood, chiefly by close personal contact with an infectious case and usually in the absence of symptoms. Large

outbreaks of hepatitis with thousands of cases have been described in South East Asia, Africa, Mexico, Central America, the Indian subcontinent, China and the former USSR. These cases were predominantly in adults between the ages of 15 and 40, and fecal contamination of water was identified as the vehicle of transmission. Serologic studies of patients in these outbreaks showed that most were already immune to HAV and that the etiologic agent was a recently described virus, HEV. The first well-documented outbreak of HEV was in New Delhi in 1955 where flood contamination of a drinking water supply apparently led to many thousands of cases of jaundice.

A commercial immunoassay for IgG and IgM antibodies to HEV was developed in 1992, and virus particles have been described based on electron microscopic examination of stool samples from infected patients [75]. The virus has also been propagated in cell culture and shown to be neutralized by patients' sera [76]. The morphologic and biophysical properties of the virus are similar to those of caliciviruses. IgG antibody persists at a reduced titer after recovery from the acute illness and appears to be protective. Where reinfection occurs it does not commonly cause serious illness and may be subclinical [77]. Acute hepatitis E is normally a self-limiting illness with features similar to hepatitis A, and a full recovery. The exception is in pregnant women, where mortality rates as high as 20% have been recorded [78].

Answers to the multiple choice questions

Q1:	a. False;	b. False;	c. True;	d. True;	e. True
Q2:	a. False;	b. True;	c. True;	d. False;	e. False
Q3:	a. True;	b. True;	c. False;	d. False;	e. True
Q4:	a. False;	b. True;	c. True;	d. False;	e. True
Q5:	a. True;	b. True.	c. False;	d. True;	e. False
Q6:	a. True;	b. False;	c. True;	d. True;	e. True
Q7:	a. True;	b. True;	c. True;	d. True;	e. True
Q8:	a. True;	b. True;	c. False;	d. False;	e. False

Further reading

For historical background the *British Medical Bulletin* 1972, volume 28, number 2, is invaluable. This issue was devoted to viral hepatitis and was written just as the prospect of widespread testing for markers of hepatitis infection was starting to become a reality. Prof. A. J. Zuckerman's book, *Human Viral Hepatitis*, published by the North-Holland Publishing Company of Amsterdam and Oxford (ISBN 0-7204-45132) and the American Elsevier Publishing Co., Inc. of New York (ISBN 0-444-10849-1) (Library of Congress Catalog No. 72-86770), contains much valuable information, including detailed clinical and pathologic descriptions of the disease. The Proceedings of the 1984 International Symposium on Viral Hepatitis entitled *Viral Hepatitis and Liver Disease*, published by Grune and Stratton, Inc. (ISBN 0-8089-1678-5) (Library of Congress Catalog No. 84-081469), is particularly useful for the sections on prophylaxis and control of hepatitis. The proceedings of an international symposium on *Prospects for control of hepatitis B* organized by SmithKline Biologicals, manufacturers of the first yeast-derived recombinant DNA vaccine, were published in the *Postgraduate Medical Journal* 1987, volume 63, supplement 2. These proceedings contain details of many of the vaccine trials with the recombinant hepatitis B vaccine. The proceedings of a workshop on HAV vaccine held in February 1994 on Marco Island, Florida were published in the *Journal of Infectious Diseases* 1995, volume 171, supplement 1, under the title 'An overview of the clinical development of hepatitis A vaccine'. Finally, the Wellcome Foundation Ltd in association with Pennine Press published a book in 1990 entitled *Interferons in the Treatment of Chronic Virus Infection of the Liver* (ISBN 1-870665-30-9) by A. L. F. Eddleston and B. Dixon.

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